

REPLACEMENT SHEET 1 / 9

Construct of Feline Thyrotropin-beta Subunit with First Intron

1 30
 5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
 met thr ala ile tyr leu met ser val leu
 31 75
 TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
 phe gly leu ala cys gly gln ala met ser phe cys phe pro thr
 76 120
 GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
 glu cys met met his val glu arg lys glu cys ala tyr cys leu
 121 162
 ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
 thr ile asn thr thr ile cys ala gly tyr cys met thr arg
Intron 1
 163 *GTATGTAGTTCATCTCACTTCTTTAGCTGAAAAATTAGATAAACCTAGACT*
CAGTCCATTCTTATCCAGAAAGGAAATGAGATAAAATCACAACCTCATTCCACAGACCTAAAC
GGTCATTGGCTCCCTAGAGGGTAGACTCCCTAGGTATATAATACGGACACTACTCCATACAG
TTGGTACAGATAATTTCACATAGTTTACTCCAAAGGTTTATTAAACCTTATCTTGTCCC
CACGATCAAGGATAAAAGAGAGGGTGTGTGTATGTCATTTCCTTGTCTTATAGGATT
CAGTGTGGATATGCTGAATTGGTATTGGGAATGGACTAAGGAATCCTCCCCAGTCCTA
TTTGTATCTATGGGATGTAAGCGAATTAACATTTGCTTCTCTTGTGCTTCCCTCAG
 580
 581 625
 GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
 asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln
 626 670
 GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
 asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile
 671 715
 CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
 pro gly cys pro his his val thr pro tyr phe ser tyr pro val
 716 760
 GCT GTA AGC TGT AAA TGT GGC AAG TGT ATAT ACT GAC TAT AGC GAC
 ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp
 761 805
 TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
 cys ile his glu ala ile lys thr asn asp cys thr lys pro gln
 806 835
 AAG TCC GAT GTG GTA GGA GTT TCT ATC TAA (GCGGCCGC₍₄₎)(AT)₅-3'
 lys ser asp val val gly val ser ile stop

() denotes the Eco RI restriction sites

Bold denotes signal sequence

Bold/italic denotes the intron 1 sequence

Fig. 1

REPLACEMENT SHEET 2 / 9

Feline Thyrotropin Alpha Subunit Construct

Fig. 2a

Fig 2b

Bold denotes 24 amino acid unexpressed signal sequence as per structure in other species

Bold italics denotes sequence upstream from expressed but not secreted signal sequence; only reported in equine; whether it is expressed is not clear.

() denotes Eco RI restriction site from TOPO Blunt vector

Underlined denotes additional sequence from TOPO Blunt vector

(₁) denotes Factor XA site

(₂) denotes Flag tag

(₃) denotes stop codon

(₄) denotes NotI restriction enzyme site

(₅) denotes extra bases needed for restriction enzyme to work

Fig. 2

REPLACEMENT SHEET 3a / 9

(GAATTG) GCCCTT

1

AGT TAC TGA GAA ATC ACA AGA CGA AGC CAA AAT CCC TCT TCA GAT

ser tyr OPA glu ile thr arg arg ser gln asn pro ser ser asp
46 90

CCA CGG TCA ACT GCC CTG ATC ACA TCC TGC AAA AAG TCC GGA GGA

pro arg ser thr ala leu ile thr ser cys lys lys ser gly gly
91 135

AGG AGA GCC ATG GAT TAC TAC AGA AAA TAT GCA GCT GTC ATT CTG

arg arg ala met asp tyr tyr arg lys tyr ala ala val ile leu
136 180

GCC ATA CTC TCT GTG TTT CTG CAT ATT CTC CAT TCT TTT CCT GAT

ala ile leu ser val phe leu his ile leu his ser phe pro asp
181 225

GGA GAG TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA
gly glu phe thr met gln gly cys pro glu cys lys leu lys glu
226 270

AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG
Asn lys tyr phe ser lys leu gly ala pro ile tyr gln cys met
271 315

GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG
gly cys cys phe ser arg ala tyr pro thr pro ala arg ser lys
316 360

AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC
lys thr met leu val pro lys asn ile thr ser glu ala thr cys
361 405

TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC
cys val ala lys ala phe thr lys ala thr val met gly asn ala

Fig. 2a

REPLACEMENT SHEET 3b / 9

406

450

AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC
lys val glu asn his thr glu cys his cys ser thr cys tyr his
451 459 492

CAC AAG ATT (ATC GAA GGT CGT₍₁₎)(GAC TAC AAG GAC GAT GAC GAT
his lys ile ile glu gly arg asp tyr lys asp asp asp asp
493 495 510
AAG₍₂₎ (TAA₍₃₎) (GCGGCCGC₍₄₎)(TATG)₅ 3'
lys

Fig. 2_b

Yoked Feline Thyrotropin

Fig. 3a

Fig 3b

() denotes the Eco RI restriction sites

Bold denotes signal sequence

Bold italics denotes intron 1 sequence 1=Factor XA site

((1)) denotes Factor XA site

((2)) denotes Flag tag

((3)) denotes stop codon

((4)) denotes Not1 restriction enzyme site

()s denotes extra bases needed for restriction enzyme to work

Fig. 3

REPLACEMENT SHEET 5 / 9

1 30
5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
met thr ala ile tyr leu met ser val leu
31 75
TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
phe gly leu ala cys gly gln ala met ser phe cys phe pro thr
76 120
GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
glu cys met met his val glu arg lys glu cys ala tyr cys leu
121 162
ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
thr ile asn thr thr ile cys ala gly tyr cys met thr arg
Intron 1
163 580 625
GTATGTAGTTCATCTCACCTCTTTAGCTGAAAATTAGATAAAACCTAGACT
CAGTCCATTCTATCCAGAAAGGAAATGAGATAAATCACACCTCATTACAG
ACCTAACGGTCATTGGCTCCTAGAGGTAGAGTCCTAGGTATAATATAACGA
CCTACTCCATAACAGTTGGTACAGATAATTTCACAATAGTTTACTCCAAAGTT
TATTTAACCTTATCTTGTGTTCCACGTCAAGGATAAAAGAGAGGTGTGTTGTT
ATGTCATTTTTTGTCTCTAGGTGATTCACTGTGGATATGCTGAATTGGTATT
GGGGAATGGGACTAAAGGAATCTCCCCCAGTCCTATTGTATCTATGGGATGT
AAGCGAATTAACATTTGCTTCTCTCTGTGCTTCCCTAG 580
581 670
GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln
626 715
671
GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile
716 760
CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
pro gly cys pro his his val thr pro tyr phe ser tyr pro val
761 805
760
GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC
ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp
761 850
806
TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
cys ile his glu ala ile lys thr asn asp cys thr lys pro gln
beta-specific primer sequence * CTP linker 850
851 892
AAG TCC GAT GTG GTA GGA GTT TCT ATC CAG GAC TCC TCT TCC TCA
lys ser asp val val gly val ser ile gln asp ser ser ser ser
CTP linker 892
AAG GCC CCT TCC GCC AGC CTT CCA AGC CCA ACG CGT CTC CCG
lys ala pro ser ala ser leu pro ser pro the arg leu pro

* reverse complement in construct

Afl III ligation site

Fig. 3a

REPLACEMENT SHEET 6 / 9

893	CTP linker	alpha-specific primer sequence	937	*										
GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TTT CCT GAT GGA GAG														
gly	pro	ser	asp	thr	pro	ile	ile	pro	gln	phe	pro	asp	gly	glu
938														977
TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA														
phe	thr	met	gln	gly	cys	pro	glu	cys	lys	leu	lys	glu		
978														1022
AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG														
Asn	lys	tyr	phe	ser	lys	leu	gly	ala	pro	ile	tyr	gln	cys	met
1023														1067
GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG														
gly	cys	cys	phe	ser	arg	ala	tyr	pro	thr	pro	ala	arg	ser	lys
1068														1112
AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC														
lys	thr	met	leu	val	pro	lys	asn	ile	thr	ser	glu	ala	thr	cys
1113														1157
TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC														
cys	val	ala	lys	ala	phe	thr	lys	ala	thr	val	met	gly	asn	ala
1158														1202
AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC														
lys	val	glu	asn	his	thr	glu	cys	his	cys	ser	thr	cys	tyr	his
1203	1211													
CAC AAG ATT (ATC GAA GGT CGT ₍₁₎)(GAC TAC AAG GAC GAT GAC GAT														
his	lys	ile	ile	glu	gly	arg	asp	tyr	lys	asp	asp	asp	asp	asp
1245	1247													1262
AAG ₍₂₎ (TAA ₍₃₎) (GCGGCCGC ₍₄₎)(TATG) ₅ 3'														
lys		*	as written											

Fig. 3_b

PEAK Expression Vector (with yoked fTSH)

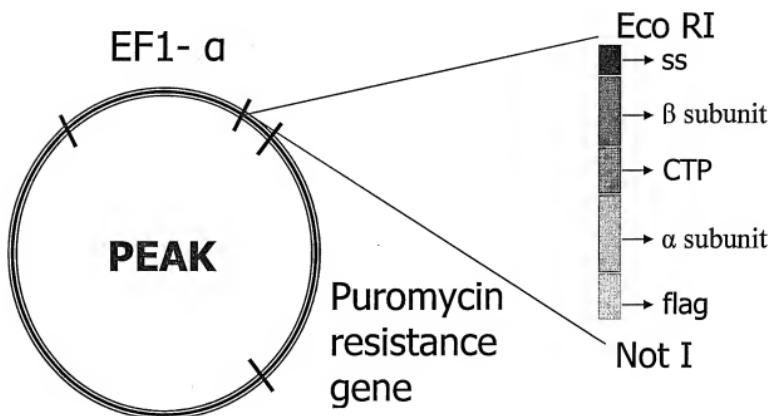


Fig. 4



Fig. 5a

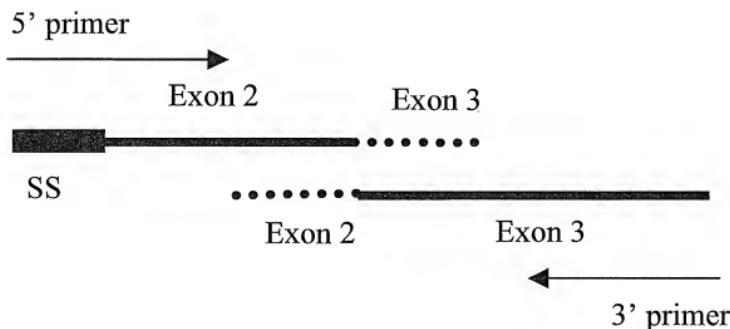


Fig. 5b

REPLACEMENT SHEET 9 / 9

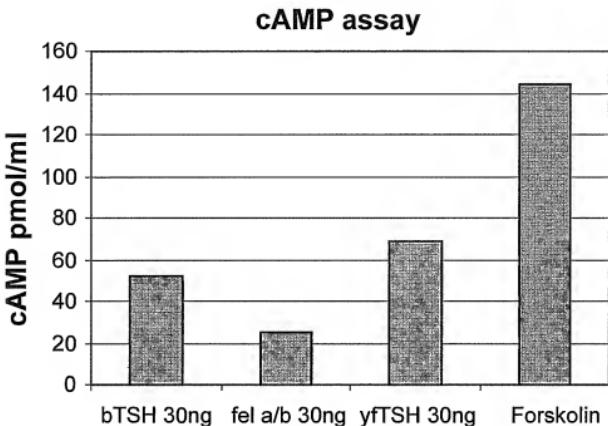


Fig. 6a

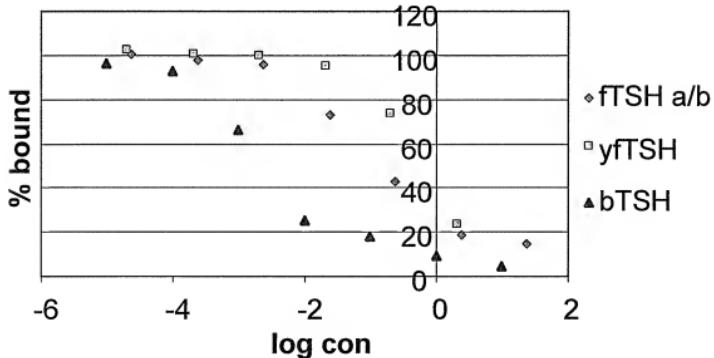


Fig. 6b